Comparative Morphology of the Early Larval Instars of *Aedes aegypti* and *A. seatoi* in Thailand

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ABSTRACT

The chaetotaxy and structures of the head, thorax and abdominal segments VIII and X of the first 3 instars of *Aedes aegypti* (Linnaeus) and *A. seatoi* Huang, are tabulated and illustrated. Characters are tabulated that will permit identification of the 4 larval stages within each species and differentiate the first 3 larval instars of *aegypti* and *seatoi*. A serial acquisition of certain thoracic setae is noted that will consistently separate second and third stage larvae of these species and at least two species in other genera. These setae will probably be very useful in deriving setal homologies, and may also represent a general character for differentiating second and third stage larvae.

INTRODUCTION

The larvae of *Aedes* (*Stegomyia*) *aegypti* (Linnaeus) and *A.* (*Stegomyia*) *seatoi* Huang can be confused easily because of similarities in their comb scales (Huang 1969), and are often collected together in certain areas of Thailand (Harrison et al. 1972). Fortunately, Huang (1969, 1972) found stable characters to separate fourth instars of these species. *Aedes aegypti* has: (1) 5 pairs of setal tufts in the ventral brush (seta 4-X), each branched; (2) meso- and metapleural spines that are thick and hooked apically; and (3) the following setal branches, 14-P (2-3), 1-VII (2) and 2-VII (single). *Aedes seatoi* has: (1) 4 pairs of setal tufts in the ventral brush (seta 4-X), each single; (2) meso- and metapleural spines thin and straight; and (3) the following setal branches, 14-P (5-9), 1-VII (5) and 2-VII (5-8).

The present study provides the following additional information about the larvae of *aegypti* and *seatoi*: (1) characters to separate the larval stages within each species; (2) characters to differentiate the first 3 larval instars of these two species; and (3) the value of the characters used by Huang, on earlier instars of these species. Since Huang (1972) summarized the fourth instar characters, they have been excluded from tables 2 and 3.

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MATERIAL AND METHODS

Study specimens came from the Bangkok and Chiang Mai strains of *aegypti* and the Sara Buri strain of *seatoi*. No differences were detected between the two *aegypti* strains. All strains were reared indoors at approximately 80°F and 80 percent relative humidity. A minimum of 25 live specimens of each larval stage were selected for each species. Selection of the original specimens to represent the 3 larval stages was accomplished by isolating individual first stage larvae shortly after hatching and selecting a given larva for mounting after the proper number of molts. Specimens were then cleared and mounted in Canada balsam or Hoyers medium for study and preparation of illustrations.

The head, thorax and last two abdominal segments were selected for study because they can be located rapidly and preliminary examinations indicated they might have significant differences. The selection of these parts does not imply a lack of differences on the abdominal segments not studied.

The use of the terms "instar(s)" and "stage(s)" follows that recommended by Anderson et al. (1971). By this interpretation "instar" means the arthropod itself, while "stage" is the term used for a period of time.

The setal numbering system employed on the illustrations (Figs. 1-6) follows that used by Belkin (1962) with minor alterations (Knight and Laffoon 1971).

RECOGNITION OF LARVAL STAGES

Characters common to both *aegypti* and *seatoi* exist that will permit separation of the 4 larval stages, but most cannot be seen unless the specimens are mounted on slides. These characters are listed in Table 1 and point out the difficulty in separating second and third instars.

TABLE 1. Characters to separate the larval stages of *aegypti* and *seatoi*

Characters	Instars			
	1st	2nd	3rd	4th
egg burster	+	0	0	0
ventral brush (seta 4-X)	0	+	+	+
seta 1-A*	+*	0*	0*	0*
seta 7-P	0	+	+	+
seta 8-M	0	0	+	+
seta 7-T	0	0	+	+
siphon sclerotization	i	i	i	c
saddle sclerotization	i	i	i	c

\+ = present i = incomplete 0* = simple
+* = with 2-3 branches 0 = absent c = complete

The siphon and saddle characters are measurements of the extent of sclerotization. Only a fourth stage larva has the siphon completely sclerotized, and saddle sclerotization extending ventrally to just above the ventral brush setae.

DIFFERENTIATION OF THE EARLY INSTARS OF *AEGYPTI* AND *SEATOI*

Both species appear to have the same setal pattern, at least on those body parts examined in this study (Figs. 1-6). Certain setae change relative position, particularly after the first molt. Seta 6-C on first instars of *aegypti* and *seatoi* is much closer to 5-C than 4-C, but on second instars it has shifted forward and is much closer to 4-C. Seta 5-C on *seatoi* apparently shifts caudally between the second and third stages. This shift was not as noticeable on *aegypti*. Setae 3 and 4-M are lateral and closer to 5-M on first instars, but on second instars 3 and 4-M have shifted mesad and 3-M is quite close to 2-M. The relative positions of 3 and 4-T seem as variable on the early instars of both species as they are on fourth instars. Siphonal seta 1-S on *aegypti* changes position in relation to the pecten. On a first or second instar of *aegypti* this seta is basal to the most distal pecten tooth while on a third instar it is often adjacent to or apical to the most distal pecten tooth. Head setae 5, 6 and 7-C are minutely barbed on the first 3 instars of *aegypti* and *seatoi*. Likewise, the antennae of the first 3 instars of *aegypti* have minute spicules, while those on *seatoi* are smooth.

Most differences in setal branching (Table 2) were small and at least partially overlapping. Setal branching on *Stegomyia* larvae can be highly variable (Rosen and Rozeboom 1954, Huang 1972), and Colless (1956) found a "hairiness factor" that could alter the size and branching of setae on *Aedes albopictus* (Skuse). Accordingly, it is best to use as many of the characters as possible. Setal branching for the first 3 instars of both species is found in Table 3.

TABLE 2. Characters to differentiate the first 3 instars of *aegypti* and *seatoi*

	1st Instar		2nd Instar		3rd Instar	
Characters*	*aegypti*	*seatoi*	*aegypti*	*seatoi*	*aegypti*	*seatoi*
seta 4-C	1	2-3	2-3	4-7	2-5	4-9
" 1-P			1-2	1-4	1-2	1-4
" 3-P					1-2	2-4
" 8-P			2-3	2-5		
" 14-P					2-3	3-5
" 14-M			2-3	3-5		
" 4-T			1-2	1-4		
" 5-T					1-2	1-4
" 13-T					2-3	3-6
" 3-VIII					2-5	2-3
" 1-S**	basal	apical	basal	apical		
" 4-X pairs			3-5	2-4	5	4

* = number of branches, except for 4-X where the number of pairs of setae are important.

** = position of 1-S in relation to the most distal pecten tooth.

TABLE 3. Setal Branching and other Characters on the First Three Instars of Aedes aegypti and A. seatoi

Seta #	1st Instar aegypti	1st Instar seatoi	2nd Instar aegypti	2nd Instar seatoi	3rd Instar aegypti	3rd Instar seatoi
SECTION A. HEAD						
1-A	2-3	2	1	1	1	1
0-C	1	1	1	1	1	1
1-C	1	1	1	1	1	1
2-C	-----not present----------				-----not present-----	
3-C	1	1	1	1	1	1
4-C	1	2-3	2-3	4-7	2-5	4-9
5-C	1	1	1	1	1	1
6-C	1	1	1	1	1	1
7-C	1	1	1	1	1	1-2
8-C	1	1	1	1	1	1
9-C	1	1	1	1	1	1
10-C	1	1	1	1	1	1
11-C	1	1	2-3	2-3	2-3	2-3
12-C	1	1	1-2	1	2-3	1-2
13-C	1	1	1	1	1	1
14-C	1	1	1	1-3	1-2	2-3
15-C	1	1	1	1	1	1
6-MP	1	1	1-2	1-2	1-2	2-3
SECTION B. PROTHORAX						
0-P	1	1	2-3	2-3	1-3	2-4
1-P	1	1	1-2	1-4	1-2	2-4
2-P	1	1	1	1	1	1
3-P	1	1	1-2	2	1-2	2-4
4-P	1	1	1-2	1-2	1-2	2-3
5-P	1	1	1	1-2	1-2	2-3
6-P	1	1	1	1	1	1
7-P	undeveloped		1	1	1-2	1-2
8-P	1	1	2-3	2-5	2-4	2-5
9-P	1	1	1	1	1	1
10-P	1	1	1	1	1	1
11-P	1	1	1-2	1-2	1-2	1
12-P	1	1	1	1	1	1-2
13-P	------not present----------				-----not present-----	
14-P	1	1	1-3	2-4	2-3	3-5
SECTION C. MESOTHORAX						
1-M	1	1	2-3	1-3	2-3	2-3
2-M	1	1	1	1	1	1-2
3-M	1	1	1	1	1	1
SECTION C. MESOTHORAX (Cont.)						
4-M	1	1	1	1	1	1-2
5-M	1	1	1	1	1	1
6-M	1	1	2	2	2-3	2-3
7-M	1	1	1	1	1	1
8-M	undeveloped		undeveloped		2-3	2-3
9-M	1	1	1	1	1-2	2
10-M	1	1	1	1	1	1
11-M	1	1	1	1	1	1
12-M	1	1	1	1	1	1
13-M	1	1	2-4	3-5	2-4	3-5
14-M	1	1	2-3	3-5	2-4	2-3
SECTION D. METATHORAX						
1-T	1	1	1-3	2-4	2-3	2-4
2-T	1	1	1	1	1	1
3-T	1	1	1-2	1-2	1-3	1-2
4-T	1	1	1-2	1-4	1-3	2-4
5-T	1	1	1-2	1-3	1-2	1-4
6-T	1	1	1	1	1	1
7-T	undeveloped		undeveloped		2-4	2-4
8-T	1	1	2-4	2-5	2-4	2-5
9-T	1	1	1	1-2	1-2	1-2
10-T	1	1	1	1	1	1
11-T	1	1	1	1	1	1
12-T	1	1	1	1	1	1
13-T	1	1	2-4	2-4	2-3	3-6
SECTION E. ABDOMINAL SEGMENTS VIII AND X						
1-VIII	1	1	2-3	1-3	2-3	2-3
2-VIII	1	1	1-2	1	1-2	1
3-VIII	1	1	1-3	1-2	2-5	2-3
4-VIII	1	1	1	1	1	1
5-VIII	1	1	1-2	1-3	1-4	1-3
C.scale	3-5	3-6	8-10	7-12	7-10	6-9
1-X	1	1	1-2	1-2	1-2	1-2
2-X	1	1	1	1	1-2	1-2
3-X	1	1	1	1	1	1
4-Xprs.	undeveloped		3-5	2-4	5	4
1-S	1	1-2	1-3	1-3	2-3	2-3
pecten	3-5	3-5	6-10	6-9	9-18	8-12

DISCUSSION

Recognition of larval stages has received the attention of many workers. Macfie (1917) recognized the presence of an egg burster and lack of a ventral brush as identifying characters for first stage larvae. The latter character has since been modified by Puri (1931) who showed that first instars of *Anopheles* have short spines on the anal segment where the brush setae will later occur. Dodge (1966) pointed out that while first stage larvae always lack a ventral brush, later stage larvae of *Wyeomyia* also lack a ventral brush. This needs further clarification, for the short spines on first instars of *Anopheles* (Baisas 1947) could certainly be called a ventral brush, and later instars of *Wyeomyia* do possess at least a single pair of setae 4-X. The bifid or trifid seta 1-A on first instars was also noted by Macfie (1917), who claimed second instars of *Stegomyia fasciata* (=*aegypti*) infrequently had this seta bifid. The latter was not observed during this study. Many *Aedes* species have seta 1-A branched in the first and later larval stages (Dodge 1963, 1966). Other species, e.g. *Ae. atropalpus* (Coquillett), have this seta single on first instars (Price 1960). Mattingly (1970) described characters for the first stage larvae of 4 species of the subgenus *Stegomyia*. Three had seta 1-A bifid or trifid, while the fourth, *Aedes woodi* Edwards, was shown with seta 1-A simple.

The extent of siphon and saddle sclerotization on first stage larvae is highly variable when examined on a generic level, but can be useful in helping to define first stage *Aedes* larvae (Dodge 1966). Often these last two characters are more valuable in differentiating third and fourth stage larvae (Macfie 1917; Belkin and McDonald 1956; Knight 1964; Smith 1965, Eddleman 1967, 1968). When using these characters care should be taken to allow for "secondary sclerotization" (Bohart 1954; Dodge 1966), which occurs during a specific stage.

The presence or absence of thoracic setae 7-P, 8-M and 7-T is useful in recognizing the first 3 larval stages, but of no value in differentiating these two species. Only 7-P is present on second stage larvae, while all 3 setae are present on third stage larvae, therefore, we consider the serial acquisition of these thoracic setae a highly reliable character resulting from the second-third stage molt. This character has a more significant potential than just the separation of two species of *Aedes*. Hurlbut (1938) described the same serial acquisition of these setae on second and third instars of *Anopheles walkeri* Theobald, and Belkin and McDonald (1956) described this sequence on *Uranotaenia anhydor* Dyar. Dodge (1964) noted a similar acquisition of 3 thoracic setae on *Toxorhynchites rutilus septentrionalis* (Dyar and Knab), and although the setae were numbered 6-P, 7-M and 7-T (Belkin 1962; Belkin et al. 1970; Knight and Laffoon 1971), they are probably homologous with 7-P, 8-M and 7-T as found on *Aedes*, *Anopheles* and *Uranotaenia*. More recently MacKenzie (1971) found that setae 8-M and 7-T appear for the first time on third instars of *Aedes* (*Aedes*) *cinereus* Meigen, *Aedes* (*Aedimorphus*) *vexans* (Meigen), *Aedes* (*Ochlerotatus*) *abserratus* (Felt and Young) and *Aedes* (*Ochlerotatus*) *atropalpus* (Coquillett). Thus, representatives from 4 of the 11 tribes of Culicinae (Belkin 1962) would have parallel serial acquisition of 3 transitory thoracic setae. If these are homologous setae, they may represent the first stable character found to differentiate second and third stage mosquito larvae in general. Bohart and Washino (1957), Knight (1964), Smith (1965, 1969), Eddleman (1967, 1968) and many others were able to separate the stages of the various species used in their studies. However,

the characters used were usually linear and meristic measurements from the head and abdominal segments VIII and X. Such characters are subject to variation caused by intrinsic and extrinsic factors, statistically valid only after the examination of a large number of specimens, and usable only on the species studied. Hopefully, future studies will give more attention to the presence or absence of setae on the thorax. Setae 7-P, 8-M and 7-T should be checked on numerous species in different genera to determine their stability for differentiating second and third stage larvae. Such a character would have wide application in many types of laboratory and field studies, and may also prove extremely useful in determining setal homologies between various genera, as well as the homologies between the thoracic chaetotaxy of the fourth stage larva and the pupal stage.

This study reveals that the first 3 instars of *aegypti* are distinct from those of *seatoi*, but like the fourth instars compared by Huang, they have many similarities. *Aedes aegypti* is a cosmopolitan member of *Stegomyia* group A (Knight and Hull 1952) which is primarily Ethiopian in distribution. *Aedes seatoi* is related to the Oriental *albopictus* subgroup of *Stegomyia* group C (Huang 1972). The similarities that exist between the larvae of these species serve notice of the problems that face taxonomists who work on *Stegomyia* larvae, especially larvae that utilize similar habitats.

Only two setal characters (14-P and 4-X) used by Huang (1969, 1972) were found useful in separating the earlier instars of *aegypti* and *seatoi*. The setae found during this study that will differentiate *aegypti* and *seatoi* in the first 3 larval stages (particularly the third), need to be checked on fourth instars. The other characters used by Huang were the thoracic pleural spines and two abdominal setae, 1-VII and 2-VII. The thoracic pleural spines on larvae in the first 3 stages are not sufficiently developed for taxonomic use. The chaetotaxy of abdominal segment VII was not studied in detail, but the two characters used by Huang were checked on third stage larvae. Setae 1-VII and 2-VII on *aegypti* are either single or double, while 1-VII on *seatoi* is single to 3-branched and 2-VII has 2-4 branches (cf. Introduction). These setae are not as valuable on third stage larvae as on those of the fourth stage.

Several chaetotaxy differences were detected that do not agree with information previously published about *aegypti*. Christophers (1960) reported setae 1, 2 and 3-P were missing on first instars of *aegypti*, while at the same time he illustrated (p. 239) two of the other first stage prothoracic setae as double, and did not label 0-P. We found all the prothoracic setae present (except 7 and 13-P) and unbranched on the first instars of *aegypti* and *seatoi*. Christophers (1960) also said 12-M and 12-T (11-M and 11-T here) were not present until the fourth larval stage. We found these setae present in all 3 early larval stages. Those on the first and second instars were minute and best seen under oil immersion. Macfie (1917) said setae 2 and 3-X are single on the first and second instars of *fasciata* (=*aegypti*), while 2-X is double on the third. We found this is usually true, but 2-X is infrequently single on third instars. Several third instars were noted with one seta 2-X single and the other double.

Recently, Pao and Knight (1970) and Knight and Laffoon (1971) have renamed seta bmh of Marshall (1938) as 6-MP. This seta is located on the palpifer (Cook 1944), an independent sclerite located caudally between the maxillary palpus and the cardostipes on fourth stage larvae. These authors consider the palpifer as part of the maxilla rather than the head capsule. On first instars of *aegypti* (Christophers 1960) and *seatoi*, a separate sclerite is not evident and seta 6-MP is on the head capsule. The palpifer first

appears on second instars. This should not be interpreted as meaning the palpifer belongs to the head capsule, because larval development is not completed until the fourth stage. Some changes in the pattern of head and mouth part sclerotization should be expected between embryonation and the fourth stage. Further studies using stains are needed to determine if a suture exists even on first instars.

CONCLUSIONS

The 4 larval stages of Thailand *Aedes aegypti* and *seatoi* can be recognized by stable characters, and these species can be differentiated in those stages. Several of the thoracic setae useful in separating the larval stages are potentially valuable tools for deriving setal homologies. The characters provided here for differentiating the earlier instars of *aegypti* and *seatoi* should be used only in laboratory studies, because none of the other species of *Stegomyia* in Thailand have the earlier instars described, and some are still not separable in the fourth larval stage. Larval identification problems in Thailand are also hampered by larval associations of several *Stegomyia* species in a single natural or artificial habitat (Harrison et al. 1972). Therefore, when feasible, basic field studies in Thailand should rear *Stegomyia* larvae to adults for precise identification.

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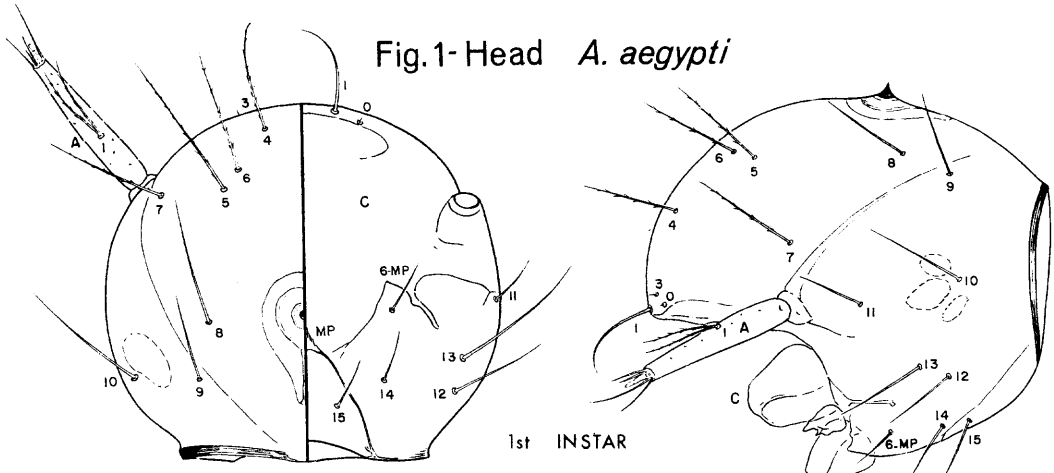
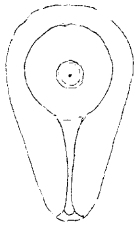
Fig. 1- Head *A. aegypti*



1st INSTAR



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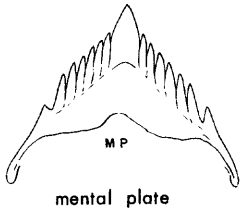

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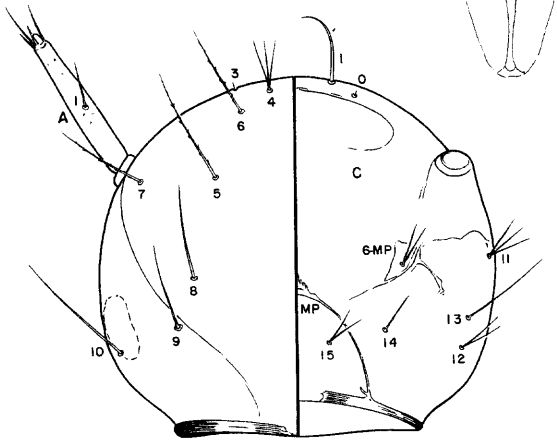

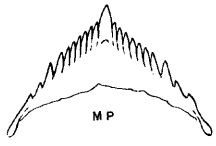

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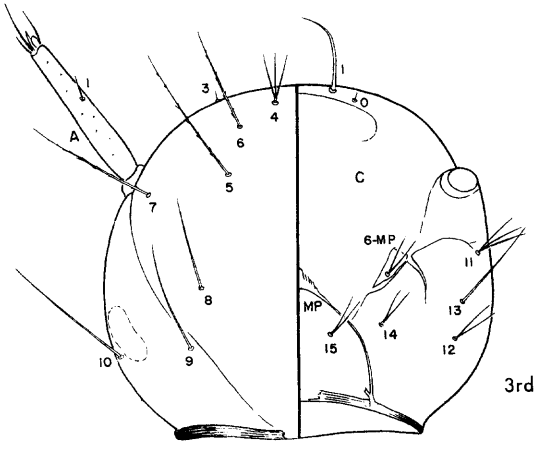
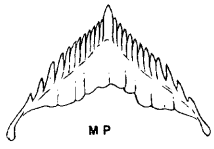




3rd INSTAR

Fig. 2 - Head *A. seatoi*

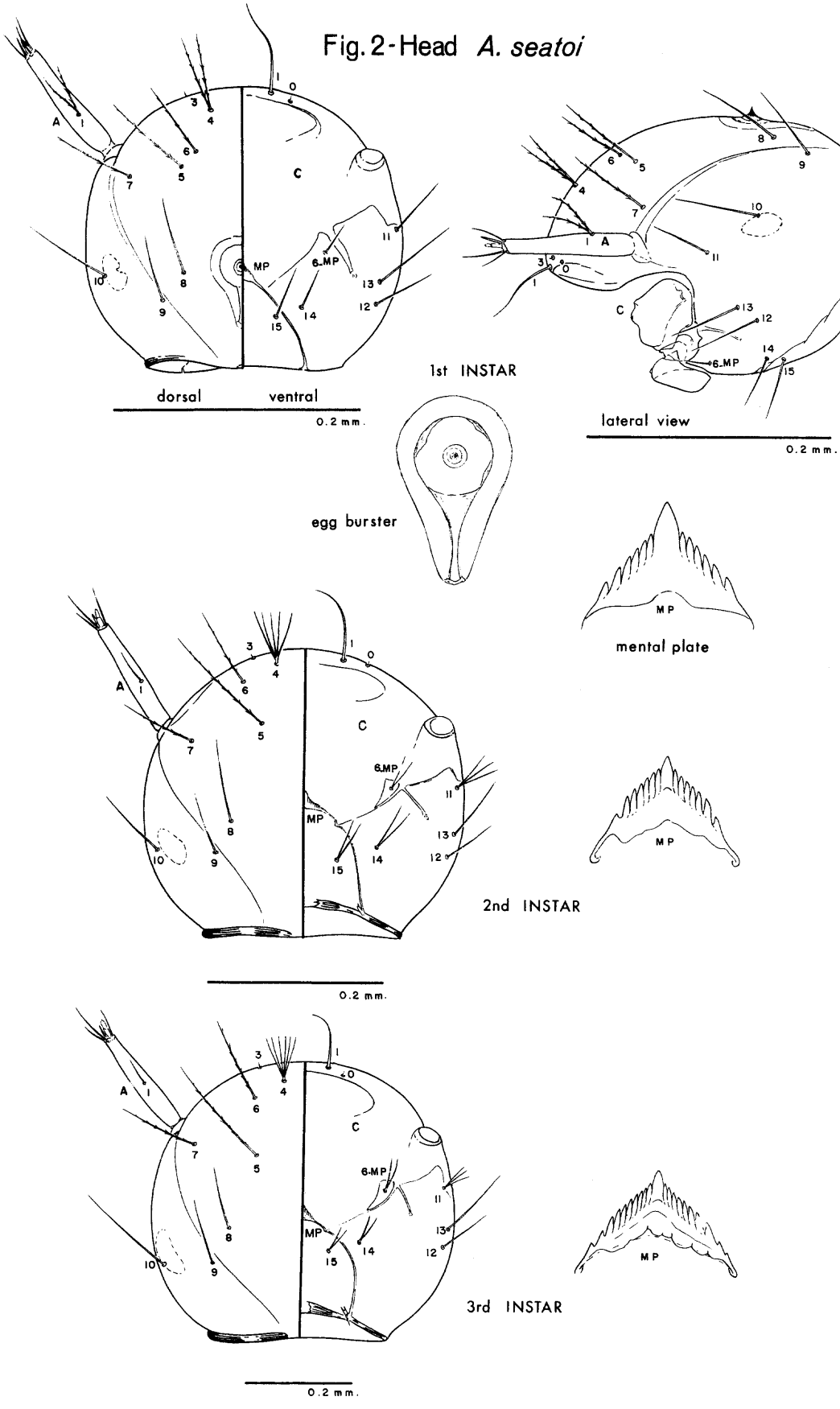


Fig. 3-Thorax *A. aegypti*

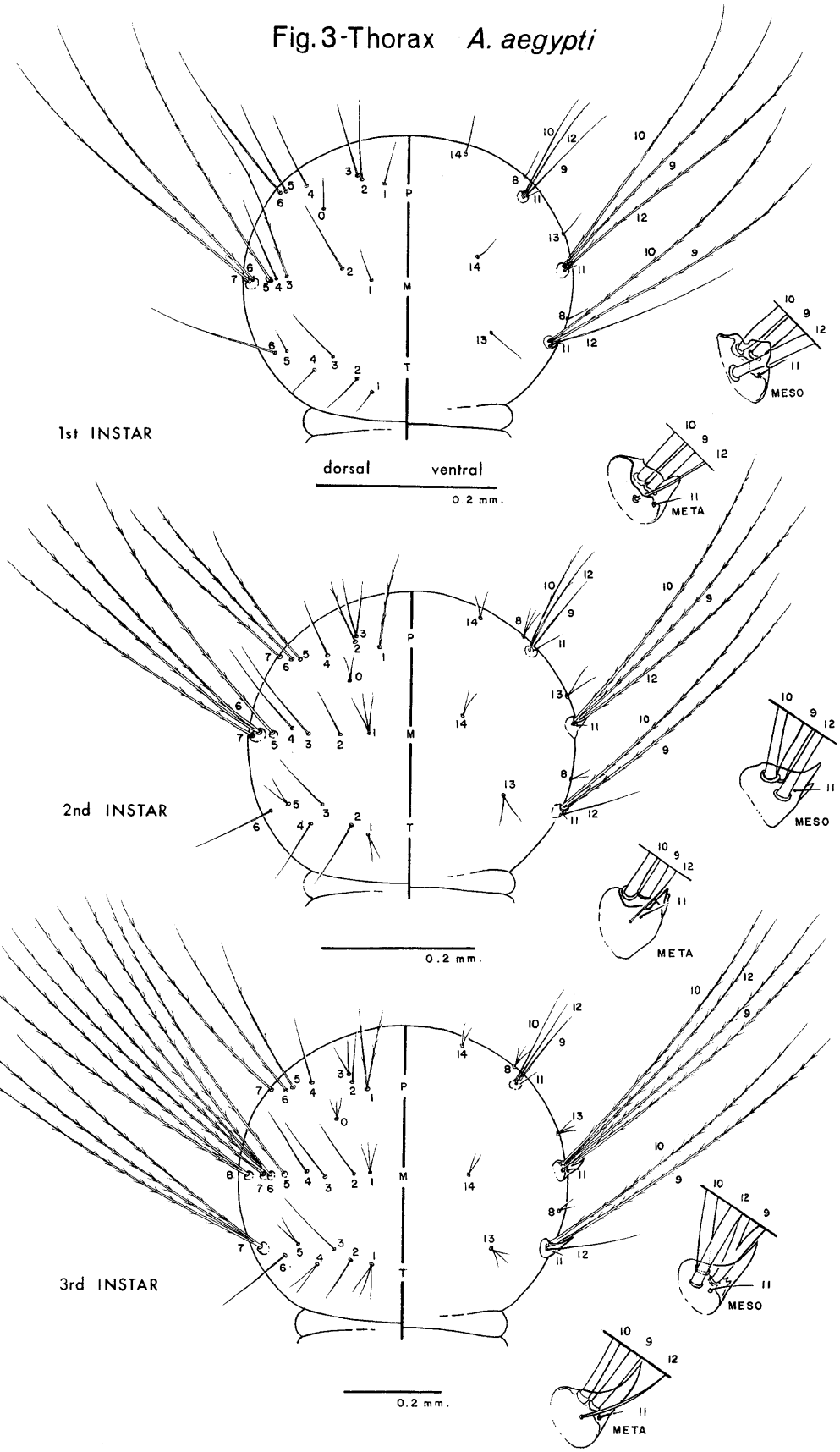


Fig. 4 - Thorax *A. seatoi*

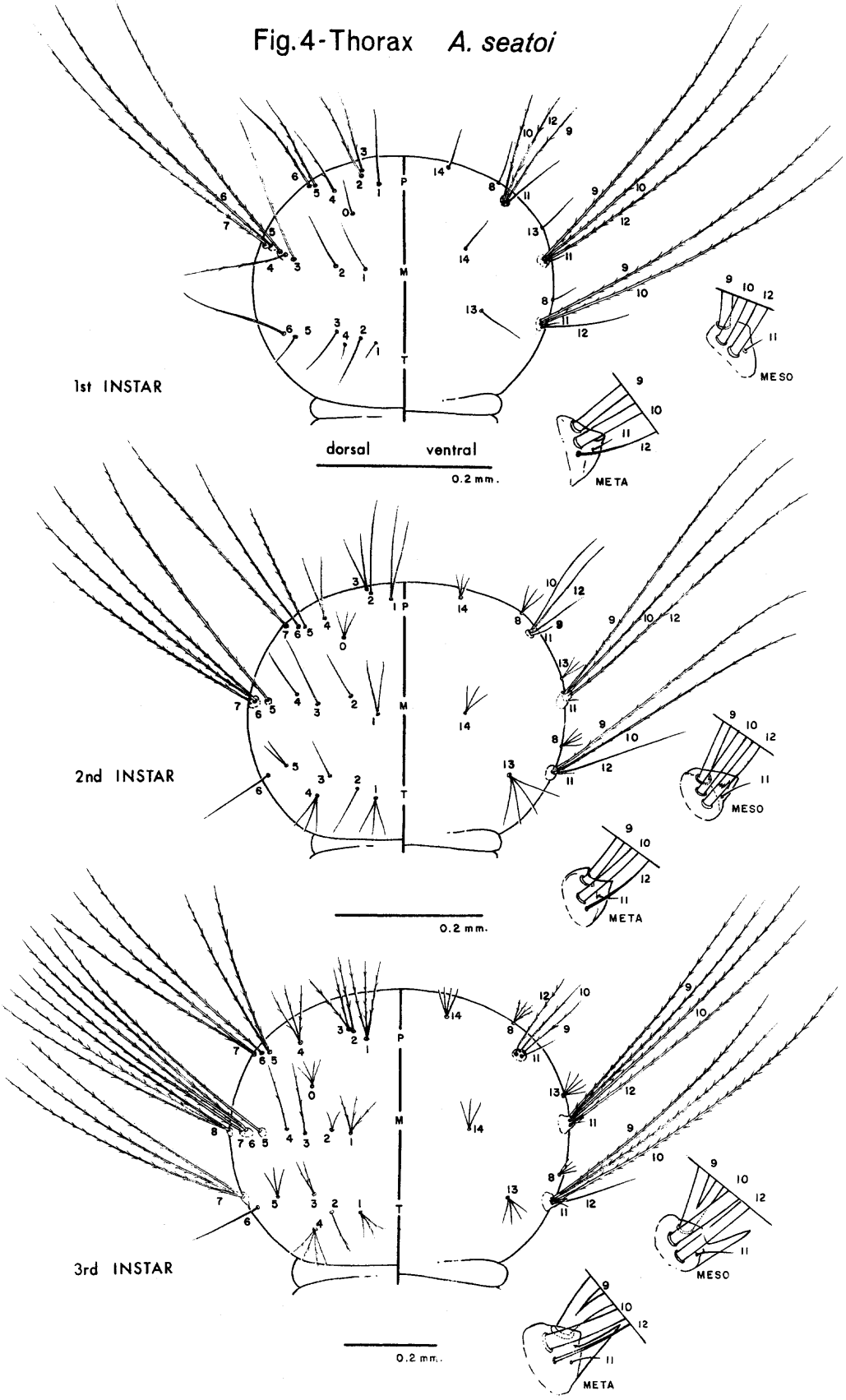
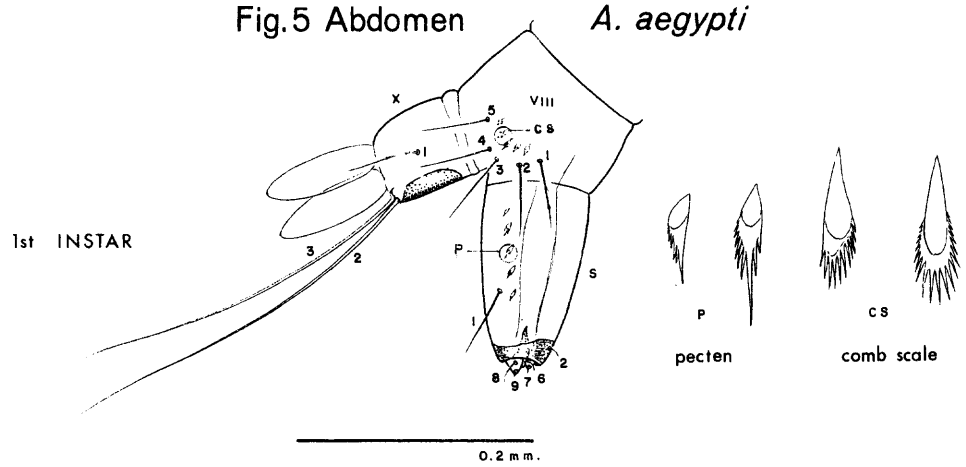
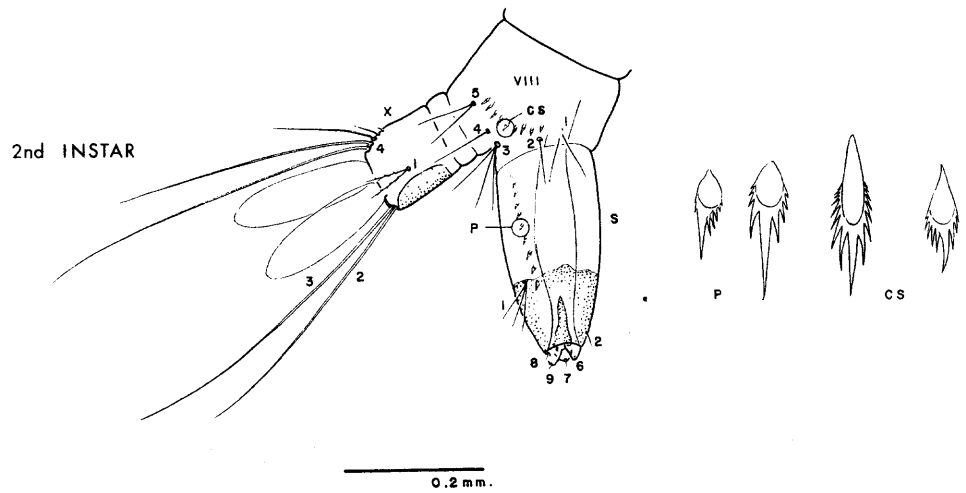


Fig. 5 Abdomen *A. aegypti*





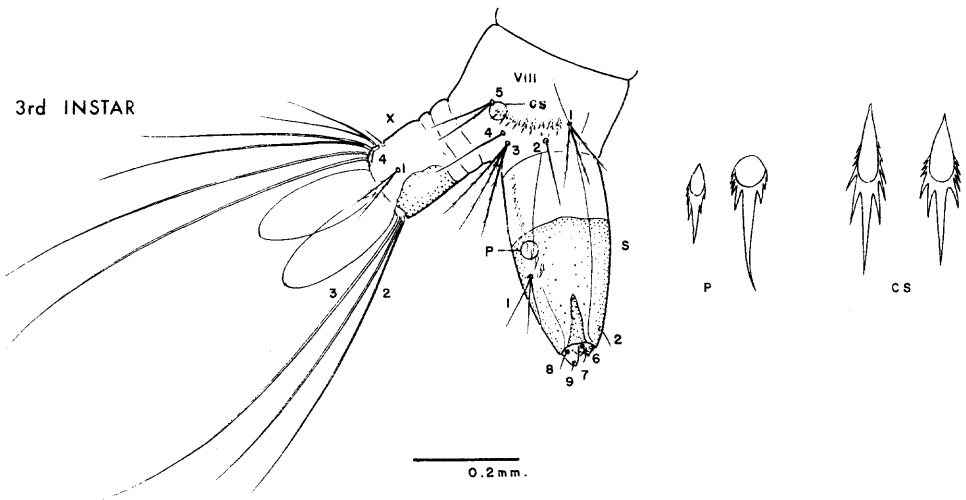


Fig.6-Abdomen *A. seatoi*